Incidence and Natural History of Mucopolysaccharidosis Type III in France and Comparison with United Kingdom and Greece

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Received 14 April 2010; Accepted 30 September 2010

Sanfilippo syndrome, or mucopolysaccharidosis type III (MPSIII) is a lysosomal storage disease with predominant neurological manifestations in affected children. It is considered heterogeneous with respect to prevalence, clinical presentation, biochemistry (four biochemical forms of the disease referred to as MPSIIIA, B, C, and D are known), and causative mutations. The perspective of therapeutic options emphasizes the need for better knowledge of MPSIII incidence and natural history. We performed parallel retrospective epidemiological studies of patients diagnosed with MPSIII in France (n = 128), UK (n = 126), and Greece (n = 20) from 1990 to 2006. Incidences ranged from 0.68 per 100,000 live-births in France to 1.21 per 100,000 live-births in UK. MPSIIIA, which predominates in France and UK, was absent in Greece, where most patients have MPSIIIB. The study confirmed the large allelic heterogeneity of MPSIIIA and MPSIIIB and detected several yet undescribed mutations. Analysis of clinical manifestations at diagnosis and over a 6–7 years follow-up indicated that almost all patients, whatever the disease subtype, expressed neurological manifestations before the age of 5 years, including language acquisition delay, cognitive delay, and/or abnormal behavior. In contrast to relatively homogeneous early onset manifestations, disease progression showed significant variation depending on subtype and age at diagnosis.

Additional supporting information may be found in the online version of this article.

Grant sponsor: Institut National de la Recherche Medicale; Grant sponsor: Institut Pasteur; Grant sponsor: Association Française contre les myopathies.

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Published online 22 December 2010 in Wiley Online Library (wileyonlinelibrary.com).

DOI 10.1002/ajmg.a.33779

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Different severities of disease progressions and different allele distribution between France and UK suggested that mutations are not equally deleterious, although genotype-phenotype correlation could not be established. Notwithstanding the rapidity of further clinical deterioration, all MPSIII patients suffer early onset devastating neurological manifestations that deserve early treatment when available. © 2010 Wiley-Liss, Inc.

**Key words:** mucopolysaccharidosis; MPSIII; sanfilippo; incidence; natural history

**INTRODUCTION**

Mucopolysaccharidosis type III (MPS III), or Sanfilippo syndrome, is a group of four autosomal recessive lysosomal storage diseases resulting from a failure to degrade glycosaminoglycan heparan sulfate. The four biochemical subtypes of MPSIII (types A–D) are caused by the deficiency of one of the four enzymes required for the removal of α-linked N-acetylgalactosamine at the non-reducing end of the saccharide chain. Heparan-N-sulfamidase (SGSH) is deficient in MPSIIIA, α-N-acetylgalactosaminidase (NAGLU) is deficient in MPSIIIB, acetylCoA:α-glucosaminide N-acetyl transferase is deficient in MPSIIIC, and N-acetylgalactosamine 6-sulfatase is deficient in MPSIIID.

Although MPSIII is a rare disease, it is with Gaucher disease and metachromatic leukodystrophy, one of the most prevalent lysosomal storage diseases. Incidence has been measured in several countries through retrospective surveys of diagnosed patients [Michelakakis et al., 1995; Meikle et al., 1999; Poorthuis et al., 1999; Applegarth et al., 2000; Pinto et al., 2004; Baehner et al., 2005; Malm et al., 2008; Lin et al., 2009]. The respective incidences of MPSIII subtypes showed large variations between countries, MPSIIIA being the most prevalent form in northern Europe, whereas MPSIIID predominates in southern countries.

MPSIII is characterized by mild somatic and severe neurological manifestations. Clinical onset, typically between 3 and 6 years, is marked by behavioral changes, with hyperactivity, aggressiveness and endangering behaviors, which evolve over a few years towards profound mental retardation and severe disability [Neufeld and Vohr, 1991; Schwartz and Muenzer, 2001; Valstar et al., 2008]. Possibly because of the low incidences, especially for MPSIIIB, C and D, and hopeless progression, available descriptions of the natural histories of the different subtypes were mostly restricted to general terms in small numbers of patients [Liem et al., 1976; van de Kamp et al., 1976, 1981; Coppa et al., 1983; van Schrojenstein-de Valk and van de Kamp, 1987; Sewell et al., 1988; Turki et al., 1989; Jansen et al., 2007; Ruijter et al., 2008]. Two retrospective studies in more than 70 diagnosed patients provided more detailed descriptions of the natural history of MPSIIIA [van de Kamp et al., 1981; Meyer et al., 2007]. Most reports emphasized clinical variability within disease subtypes, including within multiplex families.

Clinical variability of MPSIII is attributed to high allelic heterogeneity [Weber et al., 1999]. High numbers of mutations have been identified in MPSIIIA, MPSIIIB, and MPSIIIC [Beeley et al., 1998; Beeley et al., 2000; Bekri et al., 2005; Bunge et al., 1997; Coll et al., 2001; Di Natale et al., 1998; Esposito et al., 2000; Lee-Chen et al., 2002; Montfort et al., 1998; Zhao et al., 1996; Schmidtchen et al., 1998; Yogalingam and Hopwood, 2001; Muschol et al., 2004; Beeley et al., 2005; Feldhammer et al., 2009a]. With the exception of a mutation associated with mild clinical expression of MPSIIIA [Meyer et al., 2008], correlations between phenotype and genotype could not be established.

Progress in genetics and biochemistry open new perspectives for early diagnosis of lysosomal storage diseases. Because defects predominate in the central nervous system, treatments based on enzyme replacement are not currently available for MPSIII. However, animal studies have indicated that intracranial enzyme delivery could be an option for MPSIIIA in the near future [Hemsley et al., 2008; Hemsley et al., 2009], and gene therapy demonstrated efficacy on neuropathology in animal models of MPSIIIA and MPSIIIB [Cressant et al., 2004; Fridali et al., 2007]. The perspective of therapeutic trials in these rare diseases emphasizes the need for reliable and quantitative markers to assess potential benefits. In complement with biomarkers, which have still to be validated in MPSIII, clinical markers of disease progression are desired. The present study was conducted with the aims to specify the incidence of the four types of MPSIII in three European countries and to provide a quantitative description of clinical events relevant to disease progression. Data were collected through a retrospective epidemiological study based on an exhaustive registry of all French cases diagnosed between 1990 and 2006. Closely related surveys have been conducted in parallel in the United Kingdom and Greece.

**PATIENTS AND METHODS**

**Diagnosis of MPSIII**

Diagnosis of MPSIII was confirmed when enzyme catalytic activity measured in lysates of peripheral blood leukocytes and/or cultured skin fibroblasts using artificial substrates specific for each enzyme was below 10% of mean values measured in normal individuals.

In France, these assays were performed in three diagnosis laboratories. The biochemical diagnosis of MPSIII types A, B, C, and D was performed in Bron (near Lyon) and Toulouse. The biochemical diagnosis of MPSIIIB was also performed in Paris (Cochin Hospital). Sequencing of the SGSH (MPSIIIA), or NAGLU (MPSIIIB) alleles was performed for 57 patients in Bron, using genomic DNA extracted from whole blood or cultured skin fibroblasts, DNA amplification of coding region by PCR and automated...
sequencing. In the UK, enzyme diagnostics are limited to five specialized laboratories. In Greece, the biochemical diagnosis of MPSIIIB was performed in Athens and enzymatic diagnosis of MPSIIIC in London. Identification of NAGLU mutations was performed in Athens.

**Patient Enrolment and Data Collection in France**

All patients diagnosed in France between January 1st, 1990 and December 31st, 2006 (a 17 years interval) were included. To exclude patients referred from abroad for diagnosis, inclusion was restricted to patient-families living in France for at least 1 year before diagnosis. The three diagnostic laboratories (Brone, Toulouse, and Paris) provided patient lists. Lists contained information about MPSIII subtype, name, date of birth, date of diagnosis, affected relatives (multiplex family), country of residence, and name of the last known physician in charge of the patient. Data completeness was regarded as being of major importance to assess disease incidence and natural variations, the list of diagnosed patients provided by diagnostic laboratories centers was compared to information provided by two family associations (Vaincre les Maladies Lysosomales and Alliance Sanfilippo), and by physicians members of the National Pediatric Neurology Society, or of the Inborn Errors of Metabolism Society. The patient list presumed to be exhaustive was then used for data collection.

Data collection was performed through physicians involved in patient care (presently for living patients, in the past for deceased patients). With the exception of one center, all contacted physicians participated in the study. Information was collected from the participating physicians without direct contact with the families. They completed anonymized forms in which predefined sets of characteristic clinical features were listed, asking for their presence at diagnosis and their dates of occurrence during follow-up (see supporting information Table I which may be found in the online version of this article). Only one person (B.H.) had access to patient identity from the forms. Quality control was performed by B.H. with the help of the two parent associations participating in the study. They relayed information from their members to B.H. Parents of deceased children were excluded from this process.

Collected data were entered into an anonymized database, the constitution of which was approved by the French “Commission Nationale Informatique et Liberté” (approval number 716470, July 31st, 2007 and April 7th, 2008). The study design was approved by the two parent associations, by the Société Française de Neurologie Pédiatrique and by the “Comité d’évaluation et de traitement des maladies lysosomales.” Parents were informed by their physician about the study, as attested by a signed consent.

Data analysis focused on the existence of six features at diagnosis and on the occurrence of eight features during follow-up. Early language was defined as the ability to associate two words. Loss of independent walking signified permanent help for walking. Cognitive delay was the perception by the family or the caregivers of cognitive difficulties. Abnormal behaviors were hyperactivity, irritability, or aggressiveness, as noticed by the parents or the caregivers. Most children in France attend nursery school after the age of 3 years and enter elementary school after the age of 6 years. School level 1 consists of the first 2 years of nursery school. School level 2 consists of the last year of nursery school and the first 2 years of elementary school. Reaching school level 2 signifies that the child attended more than 2 years of nursery school.

**Description of Parallel Studies in the United Kingdom and Greece**

In the United Kingdom, for practical reasons, the patients’ list was established by the Society for Mucopolysaccharide diseases (UK MPS society). It included patients born (instead of diagnosed as in France and Greece) between 1990 and 2006. The UK MPS society considers that this list includes more than 90% of the families with an affected child living in the UK. Information relative to characteristic clinical features was collected by the society, through contacting parents for a telephone interview. In the case of a deceased child, information was restricted to that available on the society’s records. Parental interviews were conducted using a pre-established form identical to the one used in France.

Inclusion and data collection in Greece were performed as in France (all patients diagnosed between 1990 and 2006). When feasible, a pre-established form identical to the one used in France was completed with information contained in medical records.

**Data Analysis**

The median age and mean age (±1 SD) at the occurrence of a selected characteristic feature was calculated in the group of patients that expressed that feature. The numbers of patients for whom information was collected, or was missing, are indicated in the text, or in the tables. Descriptive data were compared using the chi-squared test or the Fischer’s exact test for proportions. The Student’s t-test was used for continuous data. Calculations were carried out using the SPSS software for Windows.

**RESULTS**

**Incidence of MPSIII**

In France, the diagnosis of MPSIII was confirmed in 128 patients during the 17-year study interval. Birth dates ranged from 1959 to 2002. We determined the numbers of diagnoses performed in children born each year between 1985 and 1994, whatever the age of the patient at diagnosis, which ranged from initial months of life (in multiplex families) to 27 years. According to the annual numbers of live-births in France, the annual incidence of MPSIII ranged 0.38–1.3 per 100,000 live-births (mean 0.73 per 100,000 live-births). Among patients diagnosed with MPSIII, 87 had MPSIIIA (68%), 18 had MPSIIIB (14%), 17 had MPSIIIC (13%), and 6 had MPSIIID (5%). Mean annual incidence for MPSIII subtypes can therefore be estimated to 0.48 per 100,000 for MPSIIIA, 0.15 per 100,000 for MPSIIIB and MPSIIIC, and 0.04 per 100,000 for MPSIIID. However, due to the low number of patients, values for MPSIIIB, C, and D are only approximates.

In the United Kingdom, MPSIII was diagnosed in 126 patients born between 1990 and 2006. The annual incidence of MPSIII for children born between 1990 and 1998 ranged 0.84–1.77 per 100,000
live-births (mean 1.21 per 100,000 live-births). MPSIIIA was diagnosed in 89 children (71%), MPSIIIB in 22 children (17%), MPSIIIC in 7 children (5%), and MPSIIID in 2 children (2%). The disease type was not determined for 6 patients (5%). In Greece, MPSIII was diagnosed in 20 patients over the 17-year study interval (1990 and 2006). None of them had MPSIIIA, 16 had MPSIIIB (80%), and 3 had MPSIIIC (15%). The disease subtype was not determined for one patient.

Considering the same 1990–1994 years, mean incidence of MPSIII was 0.68 per 100,000 live-births in France, 0.97 per 100,000 in Greece, and 1.16 per 100,000 in the UK (Table I). Incidence of MPSIIIA in the UK therefore appears 1.8-fold higher than in France, and equivalent to Germany [Baehner et al., 2005]. Incidence of MPSIIIB in Greece was 6.4-fold higher than in France, or UK, with a strong predominance of MPSIIIB, as previously described [Michelakakis et al., 1995].

**Description of the MPSIII Patient Population in France**

Clinical information was obtained for 111 MPSIII patients among 128 patients diagnosed in France between 1990 and 2006 (87%; MPSIIIA, n = 76; MPSIIIB, n = 16; MPSIIIC, n = 13; MPSIIID, n = 6). Seventeen patients were lost to follow-up, cannot, or did not give consent to be enrolled in the study. Thirty enrolled children belong to multiplex families (27%). Among 96 families, 15 were multiplex (16%; MPSIIIA, n = 10; MPSIIIB, n = 2; MPSIIIC, n = 2; MPSIIID, n = 1).

**Mutations and Residual Enzyme Activity**

Among MPSIIIA patients, 46/76 (61%) showed residual SGSH catalytic activity in enzyme assay with artificial substrate. Residual activity was always lower than 10% of normal controls. Mutations were identified in 40 children with MPSIIIA, belonging to 32 families. Thirty-eight different mutations were found in MPSIIIA French patients, among which 17 had not been previously reported (see supporting information Table II which may be found in the online version of this article). The most frequently detected mutated alleles were p.S66W (16%, 4 families) [Blanch et al., 1997; Weber et al., 1997], p.R245H (21%, 8 families) [Blanch et al., 1997; Weber et al., 1997], and c.1079delC (46%, 11 families) [Weber et al., 1997]. Homozygosity was found in 21 children (34%).

Residual enzyme activity was not reported in MPSIIIB, C, and D subtypes. Mutated alleles were identified in three MPSIIIB patients: one carried mutations p.D312N (novel mutation) and p.R565Q [Bunge et al., 1999], one was homozygous for p.R482w and one carried mutations p.A582P and 384-3C>G.

**Patient Characteristics at Diagnosis**

Table II indicates the main characteristics of patients at the time of diagnosis. The median age at diagnosis was equivalent for MPSIIIA and MPSIIIB but significantly older for MPSIIIC (MPSIIIC vs. MPSIIIA P < 0.001 and vs. MPSIIIB P < 0.003). Coarse features, hepatomegaly, language delay, abnormal behavior, and epilepsy were noticed at diagnosis with equivalent frequencies in the four MPSIII subtypes. Despite older age at diagnosis, autism-spectrum disorders were slightly less frequent at diagnosis in MPSIIIC than in MPSIIIA or MPSIIIB patients, although differences were not significant, possibly due to low numbers.

In MPSIIIA patients diagnosed before the age of 5 years (37 among 73, three patients from multiplex families diagnosed in the neonatal period were excluded from the analysis), abnormal behavior (25/37, 67%) and autism-related symptoms (7/37, 19%) were less frequently noticed at diagnosis than in patients diagnosed at older age (31/36, 86% and 15/36, 42%; P = 0.06 and P = 0.03, respectively). Residual SGSH enzymatic activity was associated with slightly, although not significantly, delayed diagnosis (6.4 ± 4.7 years vs. 4.9 ± 3.8 years, P = 0.10). Autism-spectrum related disorders at diagnosis were more frequent when residual SGSH activity was detected (8/46, 39%) than when it was not (4/30, 13%, P = 0.01). Considering only children older than 5 years at diagnosis, a similar trend was seen although the difference was not significant (12/24 vs. 3/12, NS).

**Occurrence of Characteristic Events During Follow-Up**

The mean duration of follow-up was 6.9 ± 4.9 years. Table III shows the median age of occurrence of relevant clinical events during this period.

All patients acquired independent walking at the normal range, whatever the MPSIII subtype. Delayed acquisition after the age of

### TABLE I. Incidence of MPSIII in France, United Kingdom, and Greece (1990–1994)

<table>
<thead>
<tr>
<th>Year of birth</th>
<th>France</th>
<th>United Kingdom</th>
<th>Greece</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of diagnoses</td>
<td>Number of births*</td>
<td>Number of diagnoses</td>
</tr>
<tr>
<td>1990</td>
<td>3</td>
<td>762,000</td>
<td>8</td>
</tr>
<tr>
<td>1991</td>
<td>6</td>
<td>759,000</td>
<td>11</td>
</tr>
<tr>
<td>1992</td>
<td>5</td>
<td>743,000</td>
<td>10</td>
</tr>
<tr>
<td>1993</td>
<td>7</td>
<td>712,000</td>
<td>9</td>
</tr>
<tr>
<td>1994</td>
<td>4</td>
<td>711,000</td>
<td>7</td>
</tr>
</tbody>
</table>

*Live-birth; sources: Institut National de la statistique et des études épidémiologiques (INSEE) and British and Greek offices of national statistics.
2.5 years was noticed for only three MPSIIIA patients. Acquisition of early language was assessed by the age at associating two words. Information was collected for 93 children (one was too young at the end of follow-up). Only 60 children (64.5%) acquired the capacity of associating two words, of these only 40 acquired this skill before the age of 3 years (43%). This performance was not significantly different between the four MPSIII subtypes. All patients developed cognitive decline and expressed abnormal behavior during follow-up. The ages at which families, or physicians, noticed the first cognitive problems and the appearance of hyperactivity, irritability, and aggressiveness did not differ significantly between the four MPSIII subtypes (Table III). Late occurrence of cognitive decline (after the age of 5 years) was noticed in a few patients of each subtype (MPSIIIA, n = 6; MPSIIIB, n = 2; MPSIIIC, n = 4; MPSIIID, n = 2). In contrast, the ability to attend school differed between subtypes. Among patients who were older than 5 years at the end of follow-up, only 15/74 children with MPSIIIA (20%) attended level 2 school during follow-up, as compared to 5/15 children with MPSIIIB (33%), 7/13 children with MPSIIIC (54%), and 2/5 children with MPSIIID (40%) (MPSIIIA vs. MPSIIIB, NS; MPSIIIA vs. MPSIIIC, P = 0.03).

Among 105 patients for whom information was collected, 57 lost independent walking during follow-up (53.2%). Conversely, 48 patients were still able to walk at the end of follow-up, among whom only five were older than 13 years (MPSIIIA, n = 2, oldest 21 years; MPSIIIB, n = 1, oldest 17 years; and MPSIIIC, n = 2, oldest 23 years). MPSIIIA patients lost independent walking at a slightly younger age than MPSIIIB patients (NS) and at a significantly younger age than MPSIIIC patients (P < 0.001, Table III). Information about loss of relational interaction during follow-up was obtained in only 55 patients (others were too young at the end of follow-up, or information was not reliable). Occurrence appeared significantly earlier in MPSIIIA than in MPSIIIC (MPSIIIA vs. IIIB, NS; MPSIIIA vs. IIIC, P = 0.002). Epilepsy was recognized in 39.5% of MPSIIIA patients (n = 30, mean age = 8.7 years, range = 3.2–13.7 years), 50% of MPSIIIB patients (n = 8, mean age = 8.8 years, range = 5.1–11.5 years), 31% of MPSIIIC patients (n = 4, range = 8.0–31.0 years), and 17% of MPSIIID patients (n = 1, 9.4 years). Deafness was detected in 43% of MPSIIIA patients (n = 33), 37.5% of MPSIIIB patients (n = 8), 23% of MPSIIIC patients (n = 4), and 50% of MPSIIID patients (n = 3). Twenty-six patients died during follow-up. The age at death did not differ significantly between subtypes, except MPSIIIC patients who lived longer (MPSIIIA vs. MPSIIIIA or MPSIIIB, P = 0.002). Three patients died before the age of 5 years (MPSIIIA, n = 2; MPSIIIB, n = 1) and 14 patients were still alive after the age of 20 (MPSIIIA, n = 9, oldest 27 years; MPSIIIB, n = 1, oldest 27 years; MPSIIIC, n = 4, oldest 37 years).

### Factors Correlating With the Date of Occurrence of Characteristic Events During Follow-Up of MPSIIIA Patients in France

The size of the MPSIIIA patient population allowed for the assessment of potential prognostic factors. The impact of the age at diagnosis was assessed by comparing patients diagnosed before or after 5 years (Table IV). Early clinical events, such as the acquisition of independent walking, the acquisition of early language and the onset of cognitive difficulties, occurred at the same age in the two groups. Evolution was nevertheless more severe in patients diagnosed before 5 years, as indicated by earlier loss of relational interaction and more frequent premature death (Table IV). Finally, 7/37 (19%) of the patients diagnosed before 5 years versus 10/36 (27.8%) of patients diagnosed at older age, reached level 2 at school (NS).

The detection of residual SGSH catalytic activity in enzyme assays was not significantly related to milder evolution, although relational interaction was lost slightly later in patients with residual activity (10.0 ± 3.9 years vs. 8.8 ± 3.4 years, NS) and death was slightly less premature (15.4 ± 3.0 years vs. 13.1 ± 5.4 years, NS). The numbers of patients with or without residual activity who reached level 2 at school were comparable (10/46 vs. 7/27, 22% vs. 26%, NS). Allelic heterogeneity and variability of disease expression prevented correlation between genotype and phenotype. Groups of patients carrying one of the most frequently detected SGSH alleles

### Table II. Age and Clinical Manifestations at Diagnosis in Followed MPSIII Patients Diagnosed and Followed Up in France

<table>
<thead>
<tr>
<th>MPSIII type and number of patients (n = 107)*</th>
<th>MPSIIIA (n = 73)</th>
<th>MPSIIIB (n = 15)</th>
<th>MPSIIIC (n = 13)</th>
<th>MPSIIID (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (years, median ± SD)</td>
<td>4.9 ± 4.4</td>
<td>4.9 ± 4.5</td>
<td>12.0 ± 6.5</td>
<td>8.2 ± 5.2</td>
</tr>
<tr>
<td>Number (and proportion in %) of diagnoses made</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 5 years and before 10 years</td>
<td>36 (49%)</td>
<td>9 (60%)</td>
<td>2 (15%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>At and after 10 years and before 15 years</td>
<td>8 (11%)</td>
<td>2 (13%)</td>
<td>7 (54%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>At and after 15 years</td>
<td>4 (5%)</td>
<td>1 (6%)</td>
<td>3 (23%)</td>
<td>1 (16%)</td>
</tr>
<tr>
<td>Clinical manifestations at diagnosis (number and proportion in %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coarse features</td>
<td>67 (92%)</td>
<td>14 (94%)</td>
<td>11 (85%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>37 (51%)</td>
<td>8 (56%)</td>
<td>5 (39%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>Language delay</td>
<td>68 (93%)</td>
<td>13 (88%)</td>
<td>12 (92%)</td>
<td>4 (66%)</td>
</tr>
<tr>
<td>Abnormal behavior</td>
<td>55 (75%)</td>
<td>10 (69%)</td>
<td>10 (77%)</td>
<td>5 (83%)</td>
</tr>
<tr>
<td>Autism spectrum disorder</td>
<td>21 (29%)</td>
<td>3 (19%)</td>
<td>1 (8%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>12 (17%)</td>
<td>2 (13%)</td>
<td>1 (8%)</td>
<td>2 (33%)</td>
</tr>
</tbody>
</table>

*Information was obtained from 111 patients. However, four patients [3 MPSIIIA, 1 MPSIIIB] from multiplex family were diagnosed very early in life and excluded from the present analysis.
(p.S66W, p.R245H, or c.1079delC) did not differ with respect to age at diagnosis, or occurrence of characteristic events during follow-up. The p.S298P mutation, which had been associated with a more benign evolution [Meyer et al., 2008], was detected in two patients. These patients maintained independent walking at 16 years and 21 years, respectively, as compared to a median of loss of independent walking of 10.2/3.8 years in the entire MPSIIIA population. However, only one of these patients reached level 2 at school, and both lost social interactions during follow-up. Intra-familial variability of disease expression was emphasized by the study of multiplex families, showing that the ages at occurrence of characteristic events differed between the two siblings in more than one-half of the families (8 among 15).

**Description of Patient Population in the United Kingdom**

In the UK, clinical information was collected for 126 MPSIII patients born between 1990 and 2006 (MPSIIIA, n = 89; MPSIIIB, n = 22; MPSIIIC, n = 7; MPSIIID, n = 2; and unknown, n = 6; see above). Forty enrolled children belong to multiplex families (31.7%), including one family with three children with MPSIIIA and five families whose first child was born before 1990. Among 108 families, 17 (13.5%) were multiplex with both children included (one set of twins), and five with first child born before 1990 (22 families, 17.5%, MPSIIIA, n = 14; MPSIIIB, n = 5; MPSIIIC, n = 2; unknown, n = 1).

Mutations were identified in 20 patients with MPSIIIA, belonging to 19 families. Twenty different mutations were found, among which nine had not been previously described (see supporting information Table II which may be found in the online version of this article). The most frequent mutation was p.R245H (18.4% of alleles) [Scott et al., 1995; Blanch et al., 1997; Weber et al., 1997]. Mutation p.S66W [Blanch et al., 1997] was detected in only two cases (5%). Mutations c.1079delC, p.G275R, and p.R74C, which were relatively frequent in France, were not found in UK. Homozygosity was found in only two children. Nine different alleles were identified in seven children with MPSIIIB, among which five mutations had not been previously described (see supporting information Table II which may be found in the online version of this article). Information about enzyme activity was not collected in the UK.
Median ages at diagnosis were younger in France (MPSIIIA, 3.5 ± 2.2 years, P < 0.001 vs. France; MPSIIIB, 3.1 ± 2.2 years; MPSIIIC, 5.1 ± 2.5 years; MPSIIID, 8.3 ± 6.6 years). Information about clinical manifestations at diagnosis was collected for 67 patients (among 126). Because of this small proportion, analyses made on this group may not be representative of the entire MPSIII population in the UK. Frequencies of language delay (85%), abnormal behavior (78%), autistic spectrum disorders (66%), and epilepsy (15%) were comparable to those described for children diagnosed with MPSIIIA in France.

The mean duration of patient follow-up was 6.0 ± 4.6 years (MPSIIIA, 6.7 ± 4.4, NS vs. France). Information about the occurrence of characteristic events during follow-up was partial, again raising questions about representativeness. The age at loss of independent walking was known for 46 patients, suggesting a more rapid evolution in this group than in France (MPSIIIA, 8.8 ± 2.6 years, P = 0.01 vs. France; MPSIIIB, 12.0 ± 3.6 years, P = 0.3 vs. France; MPSIIIC, 8.8 ± 4.8 years, P = 0.04 vs. France). Among 36 patients still able to walk at the end of follow-up, seven were older than 13 years (oldest 17 years). Among patients who were older than 5 years at the end of follow-up, 4/39 patients with MPSIIIA (10%) and 5/22 patients with MPSIIIB (23%) were able to attend level 2 school, as compared to 20% and 33%, respectively, in France. Twenty-four patients died during follow-up (MPSIIIA, n = 19; MPSIIIB, n = 1; MPSIIIC, n = 1; unknown, n = 3). Considering MPSIIIA patients, death occurred more prematurely in the UK than in France (median 12.7 ± 2.9 years vs. 15.4 ± 4.1 years, P = 0.03). These results suggest a slightly more severe evolution of the MPSIII disease in UK than in France.

As observed for MPSIIIA in France, diagnosis of this disease before the age of 5 years was associated with a more severe evolution. Independent walking was lost earlier (8.7 ± 2.4 diagnosis before 5 years vs. 11.4 ± 3.0 diagnosis after 5 years, P = 0.1) and death was more premature (12.5 ± 3.0 years diagnosis before 5 years vs. 15.0 ± 0.7 years diagnosis after 5 years, P = 0.02).

### Description of Patient Population in Greece

In Greece, there were no multiplex families. Molecular analysis was performed for 15/16 MPSIIIB patients (see supporting information Table II which may be found in the online version of this article). As previously reported [Beesley et al., 2004], mutation p.Y140C was

<table>
<thead>
<tr>
<th>Occurring events</th>
<th>Age at diagnosis</th>
<th>Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 5 years (n = 37)</td>
<td>≥ 5 years (n = 36)</td>
</tr>
<tr>
<td>Independent walking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age (years ± SD)</td>
<td>1.4 ± 0.4</td>
<td>1.2 ± 0.4</td>
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<tr>
<td>Mean (years)</td>
<td>1.4</td>
<td>1.3</td>
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<tr>
<td>Number of events</td>
<td>37</td>
<td>32</td>
</tr>
<tr>
<td>Two-word combination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age (years ± SD)</td>
<td>3.4 ± 1.2</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>Mean (years)</td>
<td>3.4</td>
<td>2.8</td>
</tr>
<tr>
<td>Number of events</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Onset of cognitive delay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age (years ± SD)</td>
<td>3.0 ± 1.2</td>
<td>3.0 ± 1.5</td>
</tr>
<tr>
<td>Mean (years)</td>
<td>2.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Number of events</td>
<td>37</td>
<td>36</td>
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<tr>
<td>Abnormal behavior</td>
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<td></td>
</tr>
<tr>
<td>Median age (years ± SD)</td>
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<td>4.6 ± 4.4</td>
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<tr>
<td>Mean (years)</td>
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<td>Number of events</td>
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<tr>
<td>Loss of independent walking</td>
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<td></td>
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<tr>
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<tr>
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<td>12.0</td>
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<td>23</td>
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<tr>
<td>Loss relational interaction</td>
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<td></td>
</tr>
<tr>
<td>Median age (years ± SD)</td>
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<td>11.8 ± 4.5</td>
</tr>
<tr>
<td>Mean (years)</td>
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<td>11.2</td>
</tr>
<tr>
<td>Number of events</td>
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<td>18</td>
</tr>
<tr>
<td>Death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age (years ± SD)</td>
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<td>16.5 ± 2.9</td>
</tr>
<tr>
<td>Mean (years)</td>
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<td>16.4</td>
</tr>
<tr>
<td>Number of events</td>
<td>4</td>
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</tr>
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</table>

*aInformation was obtained from 76 patients. However, three patients from multiplex family were diagnosed very early in life and excluded from the present analysis. The mean follow-up was identical in the two studied groups of patients [6.6 ± 4.9 years for children diagnosed before the age of 5 and 7.0 ± 4.8 for other children, NS.]*
the most frequently NAGLU allele detected (16/29 detected alleles) followed by p.H414R (8/29) and p.R626X (3/29). Five patients were homozygous.

The age at diagnosis of MPSIIIB (4.1 ± 6.9 years, n = 16) did not differ with France (4.9 ± 4.4 years, n = 15). Diagnoses of three patients with MPSIIIC were made earlier than in France (4.9 ± 4.0 years, vs. 12.0 ± 6.5). The mean follow-up was 4.8 ± 5.1 years. Little information about the clinical status of patients diagnosed in Greece was collected, preventing comparison with France and the UK. We know that among the 16 patients diagnosed with MPSIIIB, none reached level 2 at school, and nine lost independent walking during follow-up (median age: 11.6 ± 6.2 years). Three MPSIIIB patients died during follow-up (3.2, 3.3, and 15 years).

**DISCUSSION**

This retrospective epidemiological study was performed with the perspective of expected advances towards early diagnosis and therapeutic approaches for MPSIII. We measured the incidence of the disease from data collected in France, UK, and Greece. The occurrence at diagnosis, or during a 6.9-year follow-up of events relevant to disease progression was analyzed in patients diagnosed in France and, when feasible, compared with similar information collected in UK and Greece, with the aim to provide a quantitative description of the natural history of the disease.

The study relies on the constitution of complete lists of patients diagnosed with MPSIII by enzymatic assay. In France, irrespectively of their date of birth, patients diagnosed during the pre-defined study period (1990–2006) were included. We presume that the number of missed patients was minimal, if any. Indeed, the biochemical diagnosis of MPSIII is performed in only three laboratories over the country. The list of diagnosed patients established according to the laboratory records was carefully cross-referenced with those provided by family associations or physicians. A similar approach was used in Greece, where a unique laboratory performs the biochemical diagnosis of MPSIII. However, completeness was not cross-referenced in this country. In the UK, the patient list was provided by the UK MPS society. It concerned patients born (instead of diagnosed) during the same study period (1990–2006). As a consequence, the studied patient population was slightly younger in UK than in France, whereas the durations of follow-up were comparable.

**Incidence of MPSIII in European Countries**

Incidence was calculated according to the number of patients born each year and then diagnosed with MPSIII before year 2006. Comparison between countries focused on years 1990–1994 with the aim to reduce the risk of missing patients with a late diagnosis. Since diagnosis may be established after the age of 15 years (Table II), especially for MPSIII C, incidence may be underestimated and mild forms of the disease under-represented. Moreover, only live-births were considered, and as antenatal diagnosis followed by elected abortion was routinely performed during the study period, calculated live-birth incidence under-estimates disease frequency.
The calculated incidence of MPSIII in France (0.68 per 100,000 live-births) was almost half that in the UK (1.15 per 100,000). The incidence in the UK was similar to values determined in Australia [Meikle et al., 1999], Germany [Baehner et al., 2005], or The Netherlands [Poorthuis et al., 1999] (Table V). In these countries, MPSIIIA was the most highly prevalent disease type, representing about two-thirds of the total MPSIII patient population. MPSIIIB was slightly more frequent than MPSIIIC, and MPSIIID was very rare. Prevalence in Greece (0.97 per 100,000 live-births) was in between France and UK. However, MPSIIIA was not diagnosed in Greece, and MPSIIIB was the most highly prevalent type, a situation similar to that previously reported for Portugal [Pinto et al., 2004] and Taiwan [Lin et al., 2009]. As previously shown [Beesley et al., 2004], a high prevalence of mutations p.Y140C, p.H414R, and p.R626X in the NAGLU gene in Greek patients indicates possible founder effects.

In combination with previously published studies performed in Germany [Baehner et al., 2005], The Netherlands [Poorthuis et al., 1999], Portugal [Pinto et al., 2004], and Sweden [Malm et al., 2008], our study completes the determination of the measured incidence of MPSIII subtypes in Europe, making data available for more than one-half of the European Union (EU) population (252.6 among 454.9 millions inhabitants, Table V). Extrapolation to the entire EU population indicates a MPSIII incidence of 1.19 per 100,000 live-births. Measured incidence values are useful to anticipate the numbers of children born every year with each type of MPSIII in the EU. This information is valuable for assessing the potential impact of newborn screening and to predict the number of patients eligible for treatments, when available. Our estimations indicate 30.4 new MPSIII patients born each year in France, Germany, Greece, The Netherlands, Portugal, Sweden, and UK (53.6 for the entire EU), including 19 patients with MPSIIIA (33.5 for the EU) and eight patients with MPSIIIB (14 for the EU).

**Characteristic Clinical Events During Disease Progression**

In addition, we collected information about a selected set of characteristic events occurring in patients before diagnosis, or during follow-up, with the aim to define indicators potentially useful to monitor disease progression. Since the study was retrospective, it suffered inherent limitations due to incompleteness and imprecision about the date of occurrence. Analyses were focused on a limited set of robust items. In France, information was obtained from 87% of diagnosed patients, who were fairly representative of the entire studied population with respect to age and duration of follow-up. Collected data were cross-referenced between the medical charts and familial remembrance and therefore are reasonably reliable. Data collected in UK and Greece were more limited. The high proportion of patients for whom part of the information was missing prevents useful analysis in these populations.

Age and clinical presentation at diagnosis were identical for MPSIIIA and MPSIIIB. The earlier diagnosis of MPSIIIA in UK as compared to France can be related to different patient enrolment methods and/or different spectrum of mutations. A vast majority of affected children showed language delay, abnormal behavior, and coarse features at diagnosis. A minority expressed autistic spectrum disorders. These observations confirm a previous report on MPSIIIA patients in Germany [Meyer et al., 2007]. The mean age at acquisition of independent walking and early language, the mean age at onset of cognitive delay, loss of independent walking, onset of abnormal behavior, and loss of relational interaction were not significantly different between MPSIIIA and MPSIIIB patients. These results indicate very similar clinical expressions of these two diseases, a conclusion that differs with the previously described more benign course of MPSIIIB [van de Kamp et al., 1981]. This latter group consists of patients from a small area of The Netherlands who appear to have a more attenuated phenotype when compared to the classical patient with MPS IIIB. Despite this difference, there was nevertheless a consistent trend for a slightly more rapid progression of MPSIIIA than MPSIIIB. Both diseases affect cognitive development early in life, presumably before 3 years, and affect early language acquisition. Whereas a large standard deviation around the median age was observed for several characteristic events, which will presumably restrict their use as disease progression indicators, cognitive delay was constant before the age of 5 years. This conclusion emphasizes the importance of cognitive evaluation during patient follow-up and of an early treatment to preventing mental degradation.

MPSIIIC patients were diagnosed at an older age than MPSIIIA or MPSIIIB patients (12.0 ± 6.5 years). Consistently, disease progression appeared slower with respect to late events, like the age at loss of independent walking, loss of relational interaction and death. In contrast, indicators of early cognitive development revealed that these children were already affected early in life, as previously noticed [Ruijter et al., 2008], with disease manifestations that did not differ from that of MPSIIIA or MPSIIIB, raising questions about conditions accounting for the delay of several years before diagnosis. Low numbers of patients with MPSIIID preclude conclusions about severity and progression of this disease subtype.

**Prognosis Factors**

MPSIIIA was more frequent in UK than in France and disease progression more rapid. These differences suggest that mutations, which partly differed between the two countries, may not be equally deleterious. However, our study confirmed previous observations indicating that allelic heterogeneity in both MPSIIIA and MPSIIIB is such that it precludes recognition of phenotype–genotype correlations that could be useful for prognosis. An exception is the p.S298P mutation of the SGSH gene, which correlates with a relatively mild form of MPSIIIA [Meyer et al., 2008]. Absence of phenotype–genotype correlations that could predict disease severity represents a serious limitation to the development of disease screening at birth. Indeed, distinction at pre-symptomatic stage of severe forms, which would fully justify invasive, high-risk and high-cost therapeutic intervention, from milder ones, for which justification of such treatments is more an issue, will become crucial when these treatments are available.

Mutations associated with residual enzyme activity could result in diseases with milder progression. Our comparison of the severity of disease progression in MPSIIIA patients showing, or not showing residual SGSH activity did not support this hypothesis, as it did
not reveal significant difference in the ages of occurrence of characteristic events. This observation suggests that residual catalytic activity measured in cell extracts using artificial substrate does not necessarily signify that functional enzyme is active in lysosomes. Inefficient targeting and rapid degradation of misfolded enzyme has been reported in several lysosomal storage diseases [Poeppel et al., 2005; Ron and Horowitz, 2008; Feldhammer et al., 2009b].

The age at diagnosis was identified as a reliable indicator of disease severity in MPSI patients [Fuller et al., 2005]. Our data indicate that diagnosis of MPSIII A before 5 years, present in 51% of the patients in France, and of MPSIII B (40% of patients), was associated with a lower proportion of children attending level 2 at school, an earlier loss of social interaction and a more premature death. The age at diagnosis therefore appears as a reliable indicator of disease severity. These data also emphasize that early diagnosis should be followed by early treatment, when available.

In the absence of genotype–phenotype correlation, and of a predictive value of residual enzyme activity, the precocity of clinical manifestations appears the best indicator of disease severity.

ACKNOWLEDGMENTS

We deeply acknowledge help from the families in France, UK, and Greece, the family associations Vaincre les Maladies lysosomales and Alliance Sanfilippo, and the French National Pediatric Neurology Society and Inborn Error of Metabolism Society. We also acknowledge the help of the following colleagues: Danegas, Béziers; M. Barth, Angers; M.A. Barthez and F. Labarthe, Tours; N. Brot, Sallanches; J. Bouloche, Le Havre; C. Cancès, Toulouse; C. Chenel, Papeete; C. Copin, Provins; J.M. Cuisset, Lille; A. David, Nantes; G. Damon, St. Etienne; D. Dobbelaere, Lille; V. Drouin-Garraud and C. Vanhulle, Rouen; M. Duval-Arnould, Creil; S. Gallet, Montluçon; B. Gilbert-Dussardier, Poitiers; P. Gouny, Vichy; P.S. Jouk, Grenoble; H. Journeil, Vannes; C. Lamy, Amiens; J.F. Lemaître, Boulogne sur Mer; M. Le Merrer, Paris; M.O. Livet, Aix en Provence; S. Odent, Rennes; L. Olivier-Faivre, Dijon; P. Parent, Brest; J.M. Pédespan, Bordeaux; S. Perelman and D. de Riciaud, Nice; A.M. Picard, Chalons sur Marne; F. Rejou, Montpellier; S. Sukno, Lille; P. Vic, Quimper; I. Zix-Kieffer, Freyming-Merlebach. This work was supported as a specific join program of the Institut National de la Recherche Medicale, the Institut Pasteur, and the Association Française contre les myopathies.

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